THE LUMINESCENCE OF A NEMERTEAN, EMPLECTONEMA KANDAI, KATO

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Introduction

A great many species in the five phyla, Plathelminthes, Nemertea, Trochelminthes, Nemathelminthes and Chaetognatha, are closely allied to one another. Among these species, no luminous form has been previously recognized. I found, however, a number of luminous nemerteans, when I visited the Marine Biological Station of the Tohoku Imperial University at Asamusi, Aomori, Japan in the summer of 1936.

These nemerteans had coiled up on *Chelyosoma*, which were collected from the bottom of Aomori Bay between Natutomari and Aburame at a depth of about 35–40 meters, and were placed in the laboratory for study. They were identified by Koziro Kato (paper in preparation) as *Emplectonema kandai* sp. nov.

It is an extraordinary fact that among so large a number of species of the five phyla, only one is found to be luminous. *Emplectonema* is a genus of the nemerteans which is widely distributed in America, the Atlantic and Pacific Oceans, the Mediterranean Sea, the White Sea and Japan. It may be expected, therefore, that more luminous species of the same genus, at least, will be observed somewhere in the future. I made some experiments on *Emplectonema kandai* during the three summers of 1936–38 at the Station mentioned above. The results are given in the present paper.

I wish to express my sincere appreciation of the facilities afforded me there by Professors S. Hatai (1937) and S. Hozawa (1938), Directors of the Laboratory. I would also like to acknowledge my indebtedness to Messrs. N. Abe, K. Atoda and K. Kato, without whose aid this paper would not have been completed.

Material

As already stated, these luminous nemerteans coiled up on *Chelyosoma*. It is necessary, therefore, to collect the latter, which are dredged (by three fishermen) from the bottom of Aomori Bay, about

[1] A preliminary note in Japanese was published in the *Rigakukai*, **35** (1937): 5–11.

15 km. off the Station. But the nemerteans are not abundant. Only six or seven individuals at best, or sometimes only one or two individuals, are obtained on about two hundred *Chelyosoma*, which are collected by the fishermen as the result of one day's work.

The nemerteans are reddish orange in color. They have many eyes. They vary in length, from 53–115 cm., and are about 0.5–0.7 mm. in diameter, when they are stretched. I found one individual 10 cm. long, but one so short is extremely rare. The female animal is readily distinguished during the summer season because it is full of eggs or enlarged gonads, but I could not distinguish the males. The animals coil up on the wall of a large vat of running sea water or on the bottom, attached by the slime which is abundantly secreted from the surface of its body (Fig. 1). They remain there quietly for two months or more, if they are not disturbed.

FIG. 1. The living and coiled whole *Emplectonema kandai* about 115 cm. long. About natural size. (Photographed by N. Abe at my request.)

The animals flash brilliantly only on stimulation. The stimulus may be mechanical, chemical, thermal or electrical. The light may appear on all parts of the body, but it disappears in one or two seconds. It is whitish green in color.

Mechanical Stimuli

The animal flashes when a glass rod or a finger is gently touched to the surface or surfaces of the coiled body. The light does not spread very far from the place or places of the contact, and lasts for only one or two seconds. Its intensity varies, depending on the strength of the contact. I thought at first that some luminous material was thrown into the sea water mixed in a slime discharged from the surface of the animal body, but this observation turned out to be incorrect.

If the coiled animal is strongly rubbed between the fingers, a brilliant light appears, but it is not observed that any luminous material comes off which adheres to the fingers. If the animal is suddenly extended, without being broken, between two hands, the

head in one hand and the tail in the other, the brilliant light also appears through the whole surface of the long body except the tip of the head. This luminescence is a most beautiful sight.

Chemical and Osmotic Stimuli

If the sea water containing the nemertean is acidified with a very dilute HCl or acetic acid, the animal gives a bright light. The acid should not be too strong, or it will kill the animal too quickly. The dead or dying nemerteans produce light continuously, until all the luminous material is probably exhausted. The addition of dilute NaOH or NH_4OH to the sea water produces the same effect, although it precipitates the Ca and Mg of the sea water.

The best way to test the luminescence of the animal is to add dilute H_2O_2 to the sea water. This action is not injurious and is reversible.

If 1 to 2 cc. each of $\frac{M}{2}$ NaCl, $\frac{M}{2}$ KCl, $\frac{M}{2}$ NH_4Cl, $\frac{M}{2}$ $MgCl_2$, $\frac{M}{2}$ $MgSO_4$, $\frac{M}{2}$ Na_2SO_4, or $\frac{M}{2}$ $(NH_4)_2SO_4$ solution are added to 100 cc. of sea water which contains a nemertean, no luminescence is observed. In 0.5 cc. $\frac{M}{2}$ $CaCl_2$ plus 100 cc. sea water, however, the animal begins to flash occasionally after about 10 minutes. The intervals of its flashing become quite regular after about 20 minutes, resembling those of a firefly. Besides these flashes, there is a very faint and continuous light in other parts of the body. If this treated animal is removed to normal sea water after about 40 minutes, it lives normally. In 1 or 2 cc. $\frac{M}{2}$ $CaCl_2$ plus 100 cc. sea water, the intervals of the flashing are very slow and somewhat irregular.

In pure $\frac{M}{2}$ KCl, $\frac{M}{2}$ $CaCl_2$ or $\frac{M}{2}$ Na_2SO_4 solution, practically isotonic with sea water, the animal gives a bright light. But if the animal is kept too long in the solution, it will be killed. A mass of slime is secreted into each solution, but no luminous material is observed in it. In pure $\frac{M}{2}$ $MgSO_4$ solution, the animal flashes after about 8 minutes. In pure $\frac{M}{2}$ NH_4Cl solution, it gives a faint light after about 20 minutes. It would seem that K, Ca, Na, Mg, or NH_4 ions cause the luminescence of the animal.

In pure $\frac{M}{2}$ NaCl or $\frac{M}{2}$ $MgCl_2$ solution, isotonic with sea water, the

animal gives no light. In pure $\frac{M}{2}$ $(NH_4)_2SO_4$ solution, also, no luminescence appears. In these cases, no cation, Na, Mg or NH_4, seems to cause any light whatever. It is a little difficult to decide from these experiments whether cation or anion stimulates the animal to become luminous since no luminescence is observed in NaCl, $MgCl_2$ or $(NH_4)_2SO_4$ solution, whereas some luminescence occurs in Na_2SO_4, $MgSO_4$ or NH_4Cl solution.

If crystals of NaCl, KCl, NH_4Cl, $CaCl_2$, $MgCl_2$, $MgSO_4$, Na_2SO_4, or $(NH_4)_2SO_4$ are added to 20 cc. of sea water, which contains the nemertean, a brilliant light is always observed. If a large amount of salt is used, the light is continuous and fades gradually, due to the death of the animal. On addition of fresh water or distilled water to the sea water, a bright light appears also. Saponin acts in the same way. The increase or decrease of osmotic pressure plays, of course, a distinct rôle in each case.

Temperature and Electrical Stimuli

If the sea water at 20° C., which contains the animal, is heated to 32–33° C., or is cooled to about 1° C., the animal produces light. With induced currents, the animal also gives light.

Luciferin and Luciferase

If the animals are placed on a heavy blotting paper, they give a bright light immediately. When they are dried over P_2O_5, light is still observed during the drying and the dried, dead animals give light when again moistened with water. When these moistened ones are dried again over P_2O_5, however, they produce no more light on being moistened again with water. The animal is slender and all parts of the body are covered by a simple, thin epithelium, where the luminous cells are located, as are the cells of other luminous animals. Evidently the luminous cells of the nemertean are not large, as the cross-section of the animal indicates (Fig. 2). This may explain why the amount of luminous material secreted by the cells is comparatively small.

The existence of luciferin and luciferase cannot be demonstrated in the usual way with either the fresh or the dried animals, which are ground with sand in a mortar and are extracted with hot or cold water. Methyl or ethyl alcohol extracts of the fresh and dried nemerteans also give no light with cold or hot water extracts. The cold water extract of the nemertean gives no light with *Cypridina luciferin*, nor does the hot water extract of the nemertean give light with *Cypridina luciferase.*

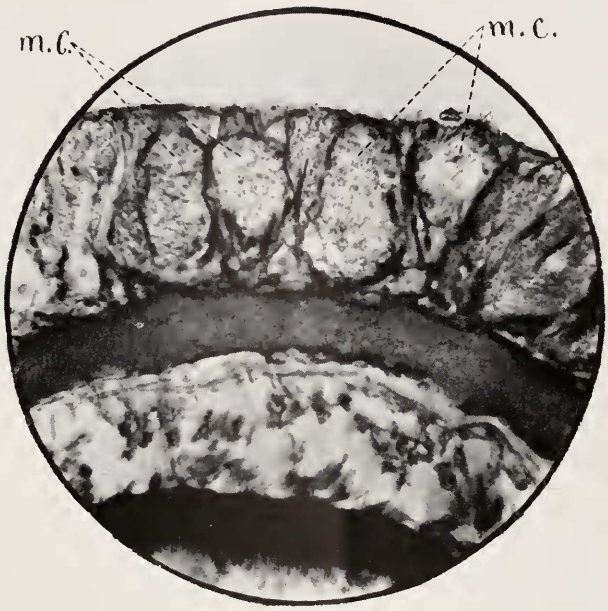
Potassium Cyanide

Since the luciferin-luciferase reaction cannot be demonstrated, the question may be asked: Is not the luminescence of the nemertean due to the symbiosis of luminous bacteria? Pierantoni (1918) holds that the light of all animals is due to symbiotic luminous bacteria. I did not attempt to raise luminous bacteria from the nemertean on an artificial culture medium, but studied the effect of KCN. According to Harvey (1921), the light of marine luminous bacteria disappears in 4 minutes, if they are treated with $\frac{M}{20}$ KCN solution, namely 0.325 per cent solution, and in 6 minutes, if treated with $\frac{M}{40}$ KCN or 0.162 per cent solution. He also shows that the light of an emulsion of the luminous organ of a fish, *Photoblepharon*, which is suspected to be symbiotic, disappears in about 20 minutes, if it is treated with 0.25 per cent KCN solution, and in about 30 minutes, if treated with 0.125 per cent KCN solution. I have found that the nemertean gives light immediately and that the light continues for about 110 minutes, if 1 cc. of the aqueous solution of $\frac{M}{2}$ KCN is added to 10 cc. of sea water which contains the animal. The animal begins to give light after 50 minutes and continues for about 140 minutes, if 0.5 cc. of $\frac{M}{2}$ KCN solution is added to 10 cc. of sea water. In both cases, the animal dies, not from dilution of sea water, but from KCN, since the worm can live indefinitely without evident injury in 100 cc. of sea water plus 20 cc. of distilled water.

These facts indicate that KCN, which inhibits most cell oxidations instantly, has very little effect on the luminescence of the nemertean. They would seem, also, to show that the light of the animal is not of bacterial origin. The failure to prove the presence of luciferin and luciferase in the animal does not necessarily indicate the symbiosis of luminous bacteria. On the contrary, I believe that luminescence in this nemertean arises from a chemical luminous material secreted in its luminous cells.

FIG. 2. Microscopic photograph of portion of a transverse section of the body surface of *Emplectonema kandai*, showing mainly the mucin-secreting cells (*m. c.*). About × 500. (Section prepared by K. Atoda at my request.)

FIG. 3. Microscopic photograph of portion of a transverse section of the body surface of *Emplectonema kandai*, showing some light-producing cells (*l. c.*) and mucin-secreting cells (*m. c.*). About × 500. (Section prepared by K. Atoda at my request.)



FIGS. 2 and 3.

Histology

I have studied the transverse and longitudinal sections of this worm, which had been kindly prepared by K. Atoda and K. Kato. The epithelium of the worm is very simple, though it is comparatively wide. In general, there appear in the epithelium, two kinds of glandular cells which stain with Delafied's haemotoxylin and eosin. Those which stain blue with haemotoxylin are large and open through the cuticle of the epithelium (Fig. 2). They are apparently the mucin-secreting

FIG. 4. Microscopic photograph of portion of a longitudinal section of the head-tip surface of *Emplectonema kandai*, where no light cells show. About × 500. (Section prepared by K. Kato at my request.)

cells. In some preparations, however, a great many cells are almost devoid of slime, which was probably discharged while the worm was being narcotized with menthol.

The cells staining with eosin appear to consist of two types, although this is not always evident. Those of one type, which stain red with eosin, though not very deeply, show a small nucleus at the base, are elongate and open through the cuticle. They are filled with granules. These cells are most common throughout all preparations

studied. The cells of the other type are especially evident when Mallory's stain is used. They stain deeply with eosin. Under a high power of the microscope they are seen to contain fine granules and in some cells their content is homogeneous. I assume that these cells are merely the young, unripe ones of the second type.

I believe that all the cells which stain in eosin are the light cells of the worm (Fig. 3). It is interesting to note that the tip of the head of the worm, where no light appears, as already stated, shows none of the eosinophil cells at all (Fig. 4.) In the head or anterior part farther from the tip, however, there appear some cells, which stain deeply with eosin and also contain the granules typical of the light cells. The number of such cells increases gradually towards the middle of the worm.

As I have already stated, the luminous material of the worm has not been observed to separate from the cuticle. But this does not mean that the glandular structure of the ducts has no opening or pore in the cuticle. On the contrary, all the ducts appear to have openings, as the sections show. The luminous secretion should be very small, however, as the light cells are also small, and the light production may take place at the instant of discharge, or the light-giving action may take place in the cells before the substances reach the openings of the ducts in the cuticle.

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Summary

The luminescence of a marine nemertean worm, *Emplectonema kandai*, living on *Chelyosoma*, is described. Light appears from the whole of the body, except a small region at the head end, in response to mechanical, chemical, thermal or osmotic stimulation. The effect of salts has been studied.

The photogenic cells are in the epithelium, stain with eosin, and appear to have openings in the cuticle, but no extracellular luminous secretion could be demonstrated. Histological sections are figured.

Luciferin and luciferase could not be demonstrated, but since KCN does not inhibit luminescence, the origin of the light is thought to be the gland cells of the worm and not symbiotic bacteria.

LITERATURE

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